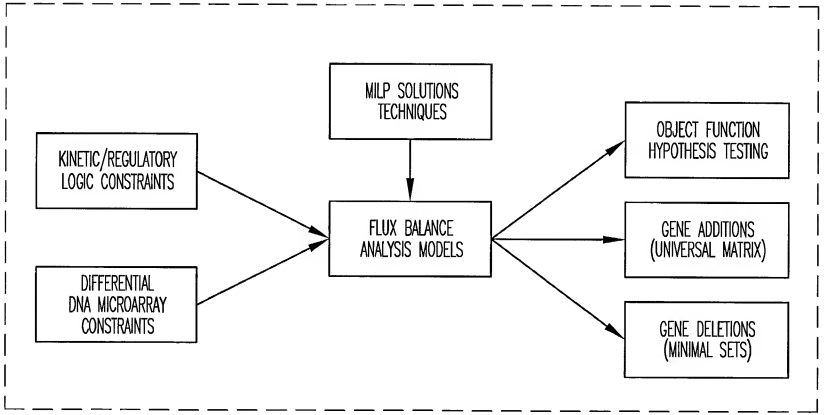


Fig. 1

Applicant: MARANAS, Costas D., et al.
Attorney Docket No. P05468US1
Filed: January 10, 2002
Title: METHOD & SYSTEM FOR MODELING CELLULAR METABOLISM
Sheet 1 of 26

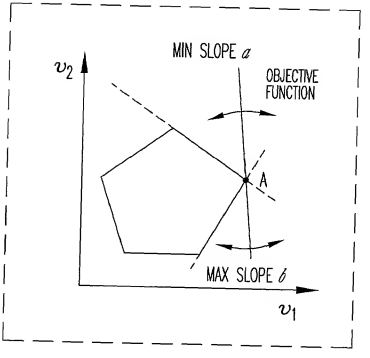

MIN SLOPE a
OBJECTIVE FUNCTION
v_2
A
MAX SLOPE b
v_1

Fig.2A

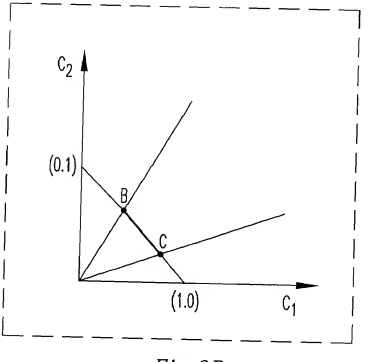

C_2
(0.1)
B
C
(1.0)
C_1

Fig.2B

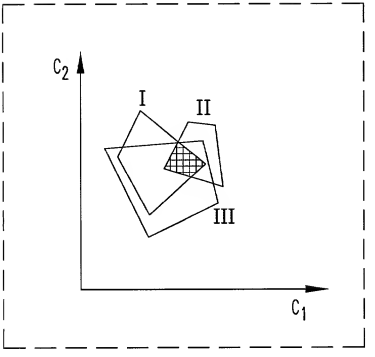


Fig. 3A

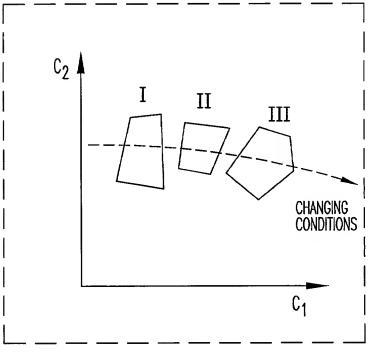


Fig. 3B

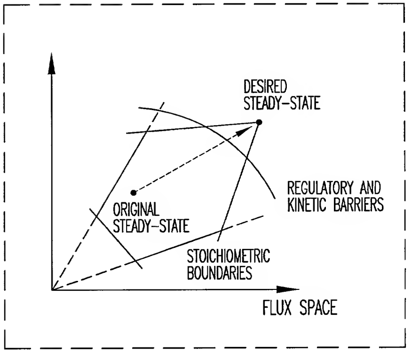

DESIRED
STEADY-STATE
REGULATORY AND
KINETIC BARRIERS
ORIGINAL
STEADY-STATE
STOICHIOMETRIC
BOUNDARIES
FLUX SPACE

Fig. 4

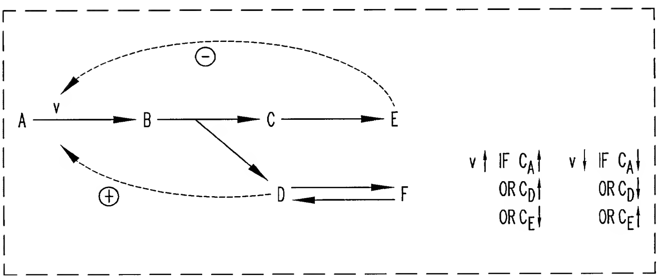

A
v
B
C
E
D
F
v ↑ IF C_A ↑
OR C_D ↑
OR C_E ↓
v ↓ IF C_A ↓
OR C_D ↓
OR C_E ↑

Fig. 5

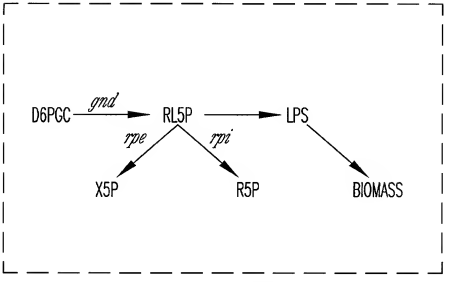


Fig. 6A

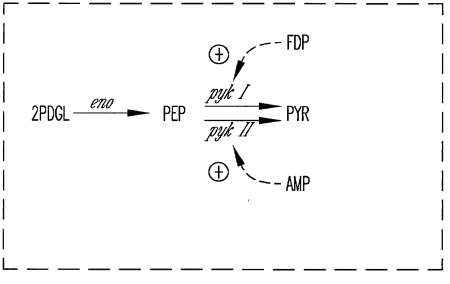


Fig. 6B

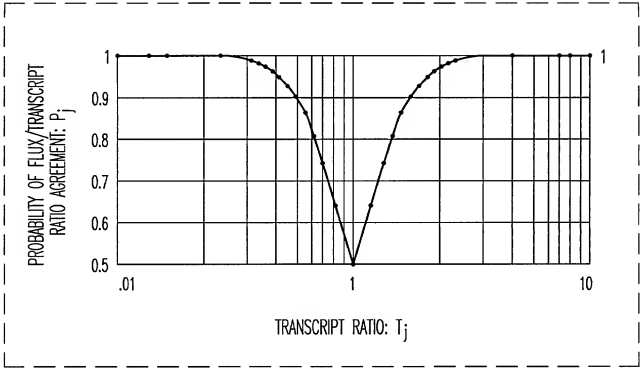

PROBABILITY OF FLUX/TRANSCRIPT RATIO AGREEMENT: P_j
1
0.9
0.8
0.7
0.6
0.5
.01
1
10
1
TRANSCRIPT RATIO: T_j

Fig. 7

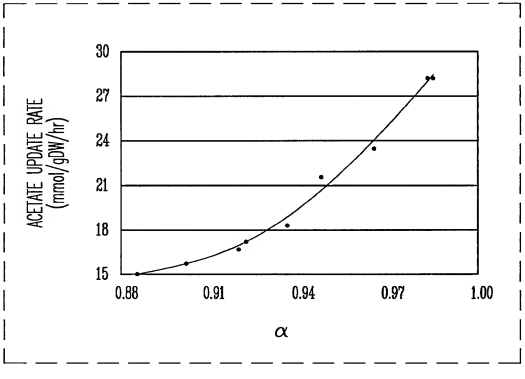

ACETATE UPDATE RATE (mmol/gDW/hr)
30
27
24
21
18
15
0.88
0.91
0.94
0.97
1.00
α

Fig. 8

MODEL PREDICTIONS OF MAXIMUM THEORETICAL YIELDS OF AMINO ACIDS FOR GROWTH ON GLUCOSE AND ACETATE

	Maximum Theoretical Yield (mmol / per 10 mmol Glucose)				Maximum Theoretical Yield (mmol / per 10 mmol Acetate)			
	Palsson '93	Modified Keasling '97	Universa l Model	% Increase	Palsson '93	Modified Keasling '97	Universal Model	% Increase
Alanine	20.00	20.00	20.00	-	3.93	5.29	5.29	-
Arginine	7.74	9.26	10.07	8.75%	1.51	2.43	2.65	9.05%
Asparagine	15.60	18.18	19.23	5.77%	3.24	4.66	4.91	5.45%
Aspartate	18.20	20.00	20.00	-	3.82	5.29	5.29	-
Cysteine	9.75	11.49	11.90	3.57%	1.81	3.29	3.42	3.80%
Glutamate	10.00	13.33	13.33	-	2.68	3.65	3.65	-
Glutamine	10.00	13.33	13.33	-	2.50	3.46	3.46	-
Glycine	20.00	35.33	35.33	-	3.94	9.00	9.00	-
Histidine	7.30	9.77	9.80	0.23%	1.37	2.43	2.54	4.53%
Isoleucine	7.34	8.00	8.07	0.91%	1.44	2.13	2.13	-
Leucine	6.67	8.00	8.00	-	1.59	2.18	2.18	-
Lysine	7.84	8.45	8.45	-	1.55	2.18	2.18	-
Methionine	5.74	7.04	7.19	2.16%	1.11	1.81	1.85	2.46%
Phenylalanine	5.29	5.76	5.76	-	1.00	1.47	1.47	-
Proline	10.00	10.91	10.91	-	2.10	2.90	2.90	-
Serine	20.00	23.04	23.04	-	3.94	5.87	5.87	-
Threonine	12.30	15.00	15.00	-	2.50	3.91	3.91	-
Tryptophan	4.14	4.67	4.73	1.28%	0.76	1.17	1.19	1.32%
Tyrosine	5.48	6.03	6.03	-	1.03	1.54	1.54	-
Valine	10.00	10.00	10.00	-	1.96	2.67	2.67	-

Palsson '93: *E coli* model proposed by Palsson (1993)
Modified Keasling '97. Modified Keasling (1997) *E. coli* model as described in text
Universal Model: Modified Keasling (1997) *E. coli* model augmented with non-*E coli* reactions compiled by the Kyoto Encyclopedia of Genes and Genomes
% Increase: Between the modified Keasling (1997) model and the Universal model

Fig. 9

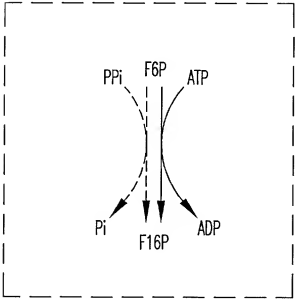


Fig. 10A

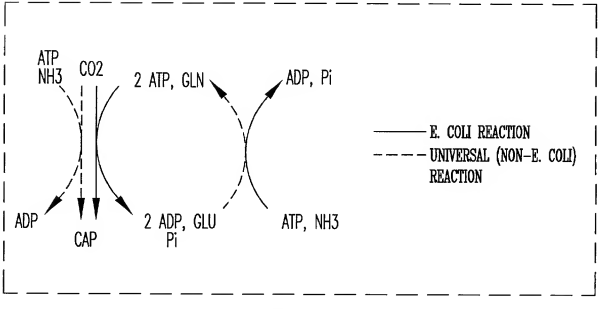


Fig. 10B

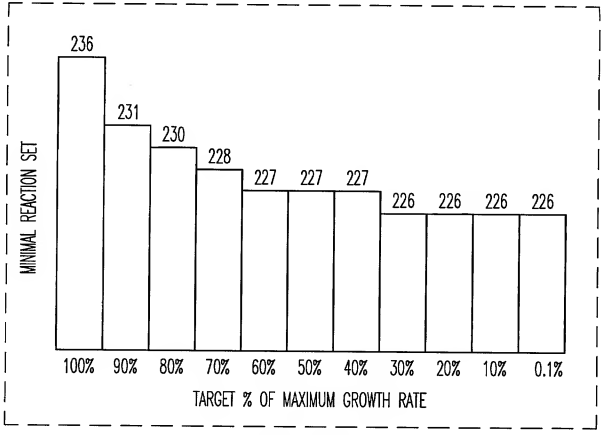


Fig. 11A

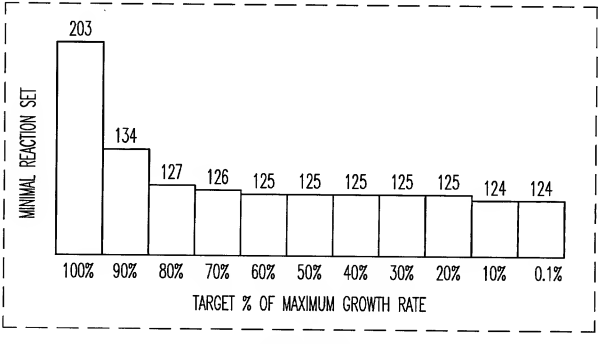


Fig. 11B

MODIFICATIONS TO THE PRAMANIK AND KEASLING MODEL*

Enzymes	Reactions
Reactions assumed irreversible	
Phosphofructokinase	Fructose-1,6-bisphosphate → Fructose-6-phosphate + Pi
Citrate Synthase	Acetyl-CoA + Oxaloacetate → CoA + Citrate
2-Ketoglutarate Dehydrogenase	2-Ketoglutarate + NAD + CoA → Succinyl-CoA + CO2 + NADH
PRSCAIM Synthetase	RCAIM + ATP + Aspartate → ADP + Pi + PRSCAIM
Glycerol Kinase	Glycerol + ATP → Glycerol-3-phosphate + ADP
Reactions removed from model	
Unknown Pathway	5'-methylthioadenosine → Adenosine + Methionine
Cystathionase	Homocysteine + Adenosine ←→ s-Adenosyl-homocystine
Sulfotransferase	Adenosine-3,5-diphosphate + sulfite ←→ 3-Phosphoadenylylsulfate
Reactions modified	
Fructose-1,6-bisphosphate Aldolase	Fructose-1,6-bisphosphate → Fructose-6-phosphate + Pi
Isocitrate Dehydrogenase	Isocitrate + NADP ←→ CO2 + NADPH + 2-Ketoglutarate
Succinate Thiokinase	Succinyl-CoA + ADP + Pi ←→ ATP + CoA + Succinate
Prephenate Dehydrogenase	Prephenate + NAD → CO2 + NADH + para-Hydroxy phenyl pyruvate
Hol Dehydrogenase	Histidinol + 3 NAD → 3 NADH + Histidine
RCAIM Synthetase	AIR + CO2 + ATP → 5-p-Ribosyl-4-carboxy-5-aminoimidazole + ADP + Pi
GTP Cyclohydrolase	GTP → D6RP5P + Formate + Ppi
3,4-Dihydroxy-2-Butanone-4-Phosphate Synthase	Ribulose-5-phosphate → DB4P + Formate
H2Neopterin Triphosphate Pyrophosphatase	AHTD → PPi + Pi + DHP
CoA Synthase	OIVAL + METTHF + NADPH + ALA + CTP + 4 ATP + CYS → THF + NADP + AMP + 2 PPi + 2 ADP + CO2 + CoA + CDP

MODIFICATIONS BASED ON INFORMATION BY KARP (1999)

Fig. 12

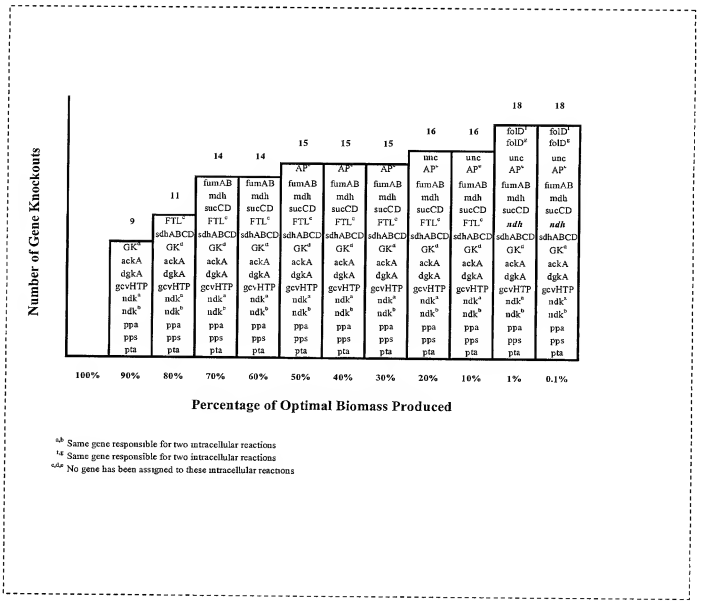

Number of Gene Knockouts
9
11
14
14
15
15
15
16
16
18
18
GK[a]
FTL[c]
fumAB
mdh
sucCD
sdhABCD
AP[c]
unc
ackA
dgkA
gcvHTP
ndk[a]
ndk[b]
ppa
pps
pta
folD[c]
folD[d]
ndh
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
1%
0.1%
Percentage of Optimal Biomass Produced
[a,b] Same gene responsible for two intracellular reactions
[c,d] Same gene responsible for two intracellular reactions
[e,f] No gene has been assigned to these intracellular reactions

Fig. 13

GENES SELECTED FOR REMOVAL BY KNOCKOUT STUDY

Enzymes	Genes	Reactions
3,5-ADP Phosphatase	AP[e]	35ADP → AMP + Pi
Acetate Kinase	ackA	AC + ATP → ACTP + ADP
CDP Kinase	ndk[a]	CDP + ATP → CTP + ADP
CMP Kinase	ndk[b]	CMP + ATP → CDP + ADP
F0F1-ATPase	unc	ADP + Pi + H_{ext} → ATP
Formate THF Ligase	FTL[c]	THF + FORMATE + ATP → ADP + Pi + FTHF
Fumarase	fumAB	FUM → MAL
Glyceraldehyde Kinase	GK[d]	GLAL + ATP → ADP + T3P1
Glycine Cleavage System	gcvHTP	GLY + THF + NAD → METTHF + NADH + CO2 + NH3
Malate Dehydrogenase	mdh	MAL + NAD → NADH + OA
Methenyl THF Cyclohydrolase	folD[f]	METHF → FTHF
Methylene THF Dehydrogenase	folD[g]	METTHF + NADP → METHF + NADPH
NADH Dehydrogenase I	ndh	NADH + Q → NAD + QH2 + 4 H_{ext}
PEP Synthase	pps	PYR + ATP → PEP + AMP + Pi
Phosphatidate Phosphatase	dgkA	DGR + Pi → PA
Phosphotransacetylase	pta	ACTP + COA → ACCOA + Pi
Pyrophosphatase	ppa	PPi → 2 Pi
Succinate Dehydrogenase	sdhABCD	SUCC + FAD → FADH2 + FUM
Succinate Thiokinase	sucCD	SUCCOA + GDP + Pi → GTP + COA + SUCC

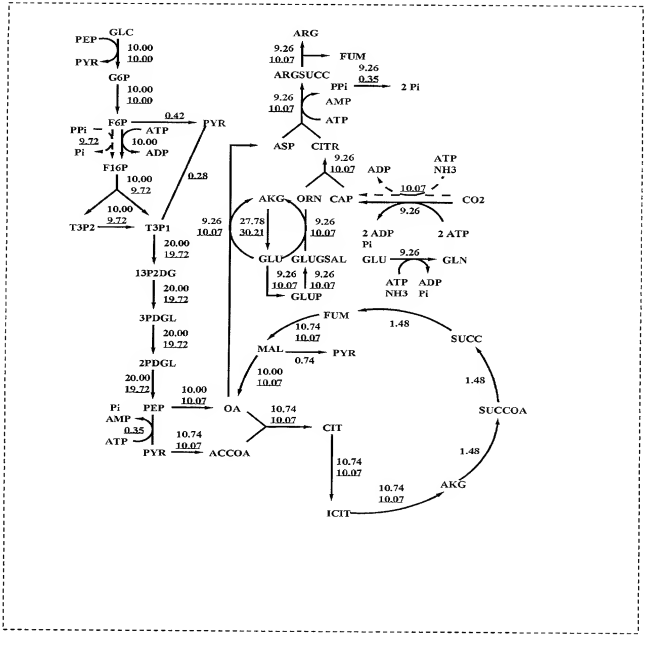
a,b Same gene responsible for two intracellular reactions
f,g Same gene responsible for two intracellular reactions
c,d,e No gene has been assigned to these intracellular reactions

Fig. 14

MODEL SELECTIONS OF ENZYMATIC REACTIONS THAT WILL ENHANCE THE AMINO ACID PRODUCTION CAPABILITIES OF *ESCHERICHIA COLI*

Amino Acid	Substrate	EC#	Enzyme	Reaction Catalyzed
Arginine	Glucose:	2.7.1.90	6-Phosphofructokinase (pyrophosphate)	Fructose-6-P + PPi → Fructose-1,6-Bisphosphate + Pi
		2.7.2.2	Carbamate kinase	ATP + NH3 + CO2 → ADP + Carbamoyl Phosphate
	Acetate:	2.7.2.2	Carbamate kinase	ATP + NH3 + CO2 → ADP + Carbamoyl Phosphate
		2.7.2.12	Acetate kinase (pyrophosphate)	Acetate + PPi → Pi + Acetyl-Phosphate
Asparagine	Glucose/ Acetate:	6.3.1.4	Aspartate—ammonia ligase (ADP-forming)	ATP + NH3 + L-Aspartate → Pi + ADP + L-Asparagine
Cysteine	Glucose/ Acetate:	2.7.7.5	Sulfate adenylyltransferase (ADP)	Sulfate + ADP → Pi + Adenylyl-Sulfate
Histidine	Glucose:	1.4.1.10	Glycine dehydrogenase	NAD + glycine → glyoxylate + NADH + NH3
		2.7.1.90	6-Phosphofructokinase (pyrophosphate)	Fructose-6-P + PPi → Fructose-1,6-Bisphosphate + Pi
	Acetate:	1.4.1.10	Glycine dehydrogenase	NAD + glycine → glyoxylate + NADH + NH3
		4.1.1.38	Phosphoenolpyruvate carboxykinase (pyrophosphate)	PPi + Oxaloacetate → CO2 + Pi + PEP
Isoleucine	Glucose:	many		
Methionine	Glucose:	2.7.7.5	Sulfate adenylyltransferase (ADP)	Sulfate + ADP → Pi + Adenylyl-Sulfate
	Acetate:	1.4.1.10	Glycine dehydrogenase	NAD + glycine → glyoxylate + NADH + NH3
		2.7.7.5	Sulfate adenylyltransferase (ADP)	Sulfate + ADP → Pi + Adenylyl-Sulfate
		2.7.9.1	Pyruvate,phosphate dikinase	Pyruvate + Pi + ATP → AMP + PPi + PEP
		4.1.1.38	Phosphoenolpyruvate carboxykinase (pyrophosphate)	PPi + Oxaloacetate → CO2 + Pi + PEP
Tryptophan	Glucose:	2.7.1.90	6-Phosphofructokinase (pyrophosphate)	Fructose-6-P + Ppi → Fructose-1,6-Bisphosphate + Pi
		2.7.9.1	Pyruvate,phosphate dikinase	Pyruvate + Pi + ATP → AMP + PPi + PEP
	Acetate:	2.7.9.1	Pyruvate,phosphate dikinase	Pyruvate + Pi + ATP → AMP + PPi + PEP
		4.1.1.38	Phosphoenolpyruvate carboxykinase (pyrophosphate)	PPi + Oxaloacetate → CO2 + Pi + PEP

Fig. 15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PEP
PYR
10.00
10.00
G6P
10.00
10.00
F6P
0.42
PYR
PPi
9.72
Pi
ATP
10.00
ADP
F1P
10.00
9.72
0.28
10.00
9.72
T3P2
T3P1
9.26
10.07
20.00
19.72
13P2DG
20.00
19.72
3PDGL
20.00
19.72
2PDGL
20.00
19.72
10.00
10.07
Pi
PEP
AMP
0.35
ATP
PYR
10.74
10.07
ACCOA
OA
10.74
10.07
CIT
ICIT
10.74
10.07
AKG
1.48
SUCCOA
1.48
SUCC
1.48
FUM
10.74
10.07
MAL
0.74
PYR
10.00
10.07
ARG
9.26
10.07
FUM
ARGSUCC
9.26
0.35
PPi
2 Pi
9.26
10.07
AMP
ATP
ASP
CITR
9.26
10.07
ADP
ATP
NH3
10.07
AKG
ORN
CAP
CO2
9.26
27.78
30.21
9.26
10.07
2 ADP
Pi
2 ATP
GLU
GLUGSAL
GLU
9.26
GLN
9.26
10.07
9.26
10.07
GLUP
ATP
NH3
ADP
Pi

Fig. 16

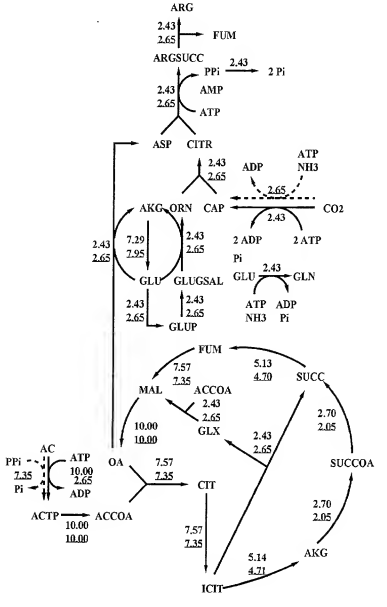
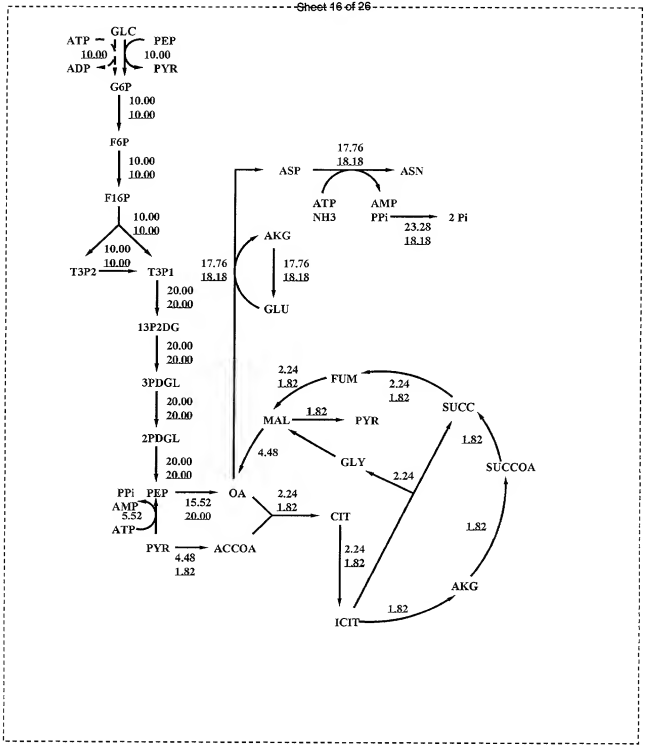

ARG
2.43
2.65
FUM
ARGSUCC
PPi
2.43
2 Pi
2.43
2.65
AMP
ATP
ASP
CITR
2.43
2.65
ADP
ATP
NH3
2.65
AKG
ORN
CAP
CO2
2.43
2.43
2.65
2 ADP
2 ATP
Pi
7.29
7.95
2.43
2.65
GLU
GLUGSAL
GLU
2.43
GLN
2.43
2.65
2.43
2.65
ATP
ADP
NH3
Pi
GLUP
FUM
7.57
7.35
5.13
4.70
SUCC
MAL
ACCOA
2.43
2.65
GLX
2.70
2.05
10.00
10.00
2.43
2.65
AC
PPi
7.35
Pi
ATP
10.00
2.65
ADP
OA
7.57
7.35
CIT
SUCCOA
ACTP
ACCOA
10.00
10.00
2.70
2.05
7.57
7.35
AKG
5.14
4.71
ICIT

Fig. 17


GLC
ATP
10.00
ADP
PEP
10.00
PYR
G6P
10.00
10.00
F6P
10.00
10.00
F16P
10.00
10.00
10.00
10.00
T3P2
T3P1
20.00
20.00
13P2DG
20.00
20.00
3PDGL
20.00
20.00
2PDGL
20.00
20.00
PPi
PEP
AMP
5.52
ATP
15.52
20.00
PYR
4.48
1.82
ACCOA
OA
2.24
1.82
CIT
2.24
1.82
ICIT
1.82
AKG
1.82
SUCCOA
1.82
SUCC
2.24
1.82
FUM
2.24
1.82
MAL
1.82
PYR
4.48
GLY
2.24
17.76
18.18
GLU
17.76
18.18
AKG
17.76
18.18
ASP
17.76
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ASN
ATP
NH3
AMP
PPi
23.28
18.18
2 Pi

Fig. 18

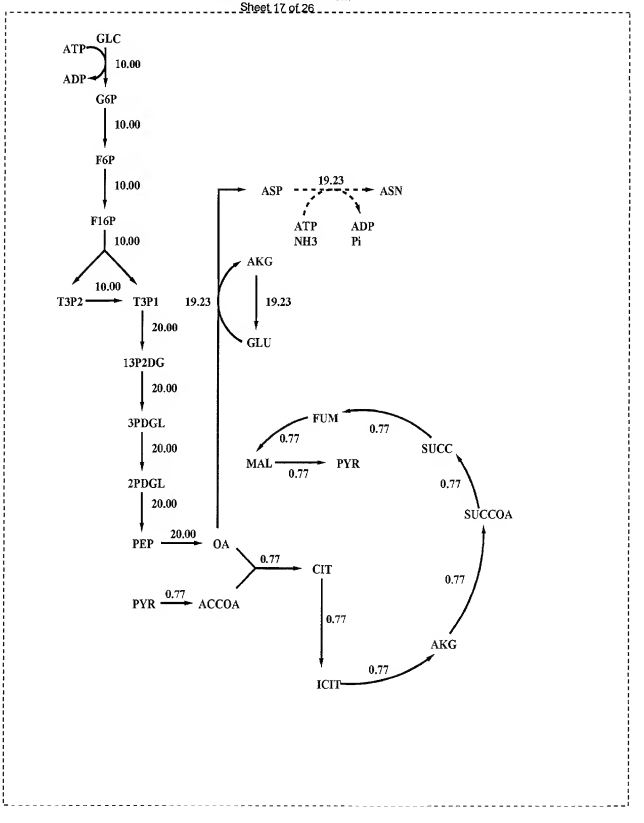
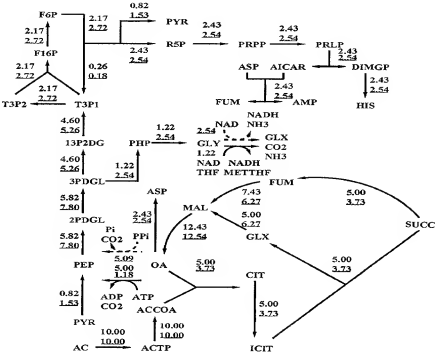

GLC
ATP
ADP
10.00
G6P
10.00
F6P
10.00
F16P
10.00
10.00
T3P2
T3P1
19.23
20.00
13P2DG
20.00
3PDGL
20.00
2PDGL
20.00
PEP
20.00
OA
ASP
19.23
ASN
ATP
NH3
ADP
Pi
AKG
19.23
GLU
FUM
0.77
0.77
SUCC
MAL
PYR
0.77
0.77
SUCCOA
0.77
CIT
PYR
0.77
ACCOA
0.77
0.77
AKG
ICIT

Fig. 19


F6P
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2.72
0.82
1.53
PYR
2.43
2.54
2.43
2.54
PRLP
2.43
2.54
F16P
2.17
2.72
R5P
PRPP
2.43
2.54
ASP
AICAR
DIMGP
0.26
0.18
2.43
2.54
2.43
2.54
FUM
AMP
HIS
2.17
2.72
T3P2
T3P1
NADH
NAD
NH3
4.60
5.26
1.22
2.54
GLX
GLY
CO2
NH3
13P2DG
PHP
1.22
NAD
NADH
4.60
5.26
1.22
2.54
THF
METTHF
3PDGL
FUM
ASP
7.43
6.27
5.00
3.73
5.82
2.80
MAL
2PDGL
Pi
2.43
2.54
5.00
6.27
SUCC
5.82
7.80
CO2
PPi
12.43
12.54
GLX
PEP
5.09
5.00
1.18
OA
5.00
3.73
5.00
3.73
CIT
0.82
1.53
ADP
ATP
CO2
ACCOA
5.00
3.73
PYR
10.00
10.00
10.00
10.00
AC
ACTP
ICIT

Fig. 20

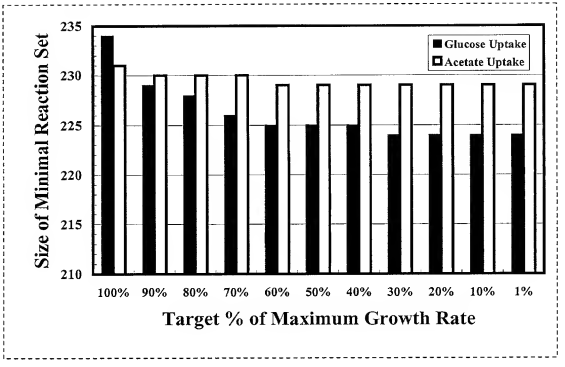
Size of Minimal Reaction Set
235
230
225
220
215
210
Glucose Uptake
Acetate Uptake
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
1%
Target % of Maximum Growth Rate

Fig. 21

EVOLUTION OF MINIMAL REACTION SETS FOR CASE (I) UNDER DECREASING GROWTH REQUIREMENTS.

Target % Maximum Growth Rate	Minimal Reaction Set (# Reactions)	Key Features
100%	234	The glycolysis, tricarboxylic acid cycle, and pentose phosphate pathways are all operating in their forward directions, optimally generating the energy cofactors ATP, NADH, and NADPH required for cell growth. All available glucose is oxidized into the cell's only secreted byproduct, carbon dioxide.
90%	229	The fluxes through two TCA cycle reactions 2-ketoglutarate dehydrogenase and succinate dehydrogenase are zero while succinyl-CoA synthetase operates in its reverse direction suggesting a less demanding energetic state under the sub-maximal growth demands. Acetate is now secreted as a byproduct along with carbon dioxide.
80%	228	Fluxes through two additional TCA cycle reactions, fumarase and malate dehydrogenase, are eliminated while a reaction secreting fumarate is added.
70%	226	The pentose phosphate pathway operates solely for nucleotide biosynthesis with the reaction fluxes through ribulose phosphate 3-epimerase, transketolase I, transketolase II, and transaldolase B all operating in reverse. Fluxes through glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase are absent in this case, replaced by pyridine nucleotide transhydrogenase which meets the cellular NADPH needs. In addition, formate is now secreted along with acetate, fumarate, and carbon dioxide.
60%, 50%, 40%	225	Acetate is no longer secreted as a metabolic byproduct, but is converted to acetyl-CoA by acetyl-CoA synthetase.
30%, 20%, 10%, 1%	224	Three glycolytic reactions, phosphoglycerate mutase, enolase, and pyruvate kinase are eliminated, but both serine deaminase and phosphoenolpyruvate synthase are added to supply the cell with phosphoenolpyruvate.

Fig. 22

METABOLITES UPTAKEN OR SECRETED AT EACH TARGET GROWTH RATE ON AN OPTIMALLY ENGINEERED MEDIUM.
U – DENOTES METABOLITE UPTAKE
S – DENOTES METABOLITE SECRETION

	Percentage of 100% Biomass Generation Required												
Metabolite	100%	99.5%	99%	98%	97%	96%	95%	90%	85%	80%	70%	60%	10%
Acetate												S	S
Acetaldehyde													U
Adenine				U	U	U	U	U	U			U	
Adenosine										U	U		U
Alanine										U	U		
Arginine	U	U	U	U	U	U	U	U	U	U	U	U	U
Asparagine									U	U		U	U
Aspartate									U	U	U	U	U
Carbon dioxide	S	S	S	S	S	S	S	S	S	S	S	S	S
Cysteine	U	U	U	U	U	U	U	U	U	U	U	U	U
D-Alanine								U	U			U	U
Thymidine		U	U	U	U	U	U	U	U	U	U	U	U
Ethanol	U	U	U	U	U	U	U	U		U		U	
Glycerol											U		
Glycerol-3-phosphate	U	U	U	U	U	U	U	U	U	U		U	U
Glutamine									U	U	U	U	U
Glutamate											S	U	U
Glycine						U	U	U	U	U	U	U	U
Guanine				U	U	U	U		U	U			
Guanosine								U			U	U	U
Histidine		U	U	U	U	U	U	U	U	U	U	U	U
Isoleucine	U	U	U	U	U	U	U	U	U	U	U	U	U
Leucine							U	U	U	U	U	U	U
Lysine	U	U	U	U	U	U	U	U	U	U	U	U	U
Meso-diaminopimelate		U	U	U	U	U	U	U	U	U	U	U	U
Methionine	U	U	U	U	U	U	U	U	U	U	U	U	U
Mannitol												U	U
Ammonia	U	U	U	U	U	U	U	U					
Oxygen	U	U	U	U	U	U	U	U	U	U	U	U	U
Phenylalanine			U	U	U	U	U	U	U	U	U	U	U
Phosphate	U	U	U	U	U	U	U	U	U	U	U	U	U
Proline					U	U	U	U	U	U	U	U	U
Putrescine	U	U	U	U	U	U	U	U	U	U	U	U	U
Pyruvate										U	U	U	U
Ribose												U	U
Serine								U	U	U	U	U	U
Spermidine	U	U	U	U	U	U	U	U	U	U	U	U	U
Threonine		U	U	U	U	U	U	U	U	U	U	U	U
Tryptophan		U	U	U	U	U	U	U	U	U	U	U	U
Tyrosine			U	U	U	U	U	U	U	U	U	U	U
Uracil						U	U	U	U	U		U	
Uridine											U	U	U
Valine							U	U	U	U	U		U
# Metabolites Uptaken	**12**	**17**	**19**	**21**	**22**	**24**	**26**	**28**	**29**	**31**	**29**	**34**	**34**

Fig. 23

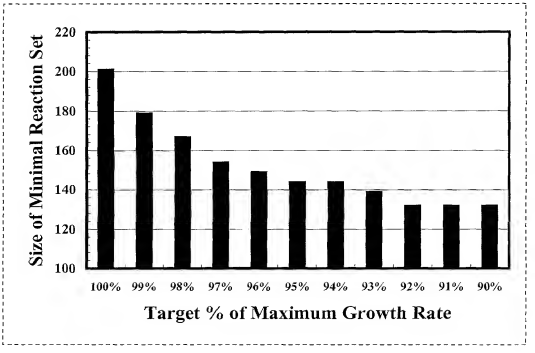
Size of Minimal Reaction Set
220
200
180
160
140
120
100
100% 99% 98% 97% 96% 95% 94% 93% 92% 91% 90%
Target % of Maximum Growth Rate

Fig. 24

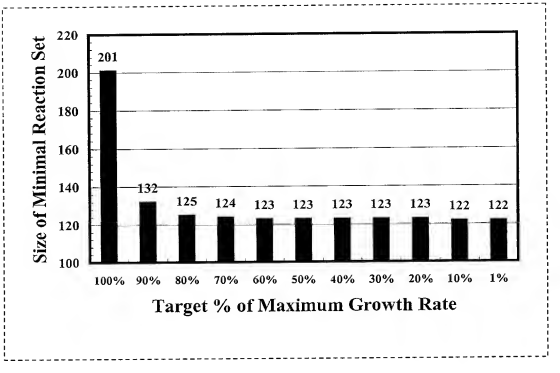
Size of Minimal Reaction Set
220
200
180
160
140
120
100
201
132
125
124
123
123
123
123
123
122
122
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
1%
Target % of Maximum Growth Rate

Fig. 25

EVOLUTION OF MINIMAL REACTION SETS FOR CASE (II) UNDER DECREASING GROWTH REQUIREMENTS.

Target % Maximum Growth Rate	Minimal Reaction Set (# Reactions)	Key Features
100%	201	The organic material transported into the cell includes ethanol and glycerol-3-phosphate which fuel glycolysis, the TCA cycle, and PPP. The flux directions of the glycolysis pathway are split with all reaction fluxes preceding glyceraldehyde-3-phosphate (G3P) dehydrogenase operating in reverse, and all fluxes following and including G3P dehydrogenase operate in their forward directions. Putrescine, spermidine, and five amino acids are transported into the network eliminating the need for biosynthetic pathways for these components.
90%	132	While the PPP and TCA cycle reactions are still functional, the network no longer utilizes the five glycolytic reactions from glyceraldehyde-3-phosphate dehydrogenase to pyruvate kinase. Consequently, the TCA cycle is completely fueled by imported ethanol and acetate rather than flux from the glycolysis pathway.
80%	125	This network tolerates the complete elimination of the TCA cycle and glyoxylate shunt. As a result, the function of the pentose phosphate pathway reactions is no longer restricted to nucleotide biosynthesis, but now includes the formation of cellular NADPH. Most of this NADPH is subsequently converted to NADH by pyridine nucleotide transhydrogenase to replace the cellular reducing power lost from the inactivity of the TCA cycle.
70%	124	A slightly less efficient set of internal metabolic reactions enables the growth demands to be met with the importation of one less metabolite (i.e. one less transport reaction) than its 80% counterpart.
60% 50%, 40% 30%, 20%	123	Neither the TCA cycle nor PPP are utilized for reducing power. Most of the cellular reducing capabilities are now generated from the uptake of ethanol and its subsequent conversion into acetyl-CoA.
10%, 1%	122	This minimal network is comprised mostly of cell envelope and membrane lipid biosynthetic reactions, along with a number of transport and salvage pathway reactions. Here, the three core metabolic routes, glycolysis, the TCA cycle, and the pentose phosphate pathway are almost completely dismantled with only one glycolytic and 4 PPP reactions remaining.

Fig. 26

FUNCTIONAL CLASSIFICATION OF MINIMAL NETWORKREACTIONS FOR GROWTH ON AN OPTIMALLY ENGINEERED MEDIUM.

Functional Classification	# rxns
ALA Isomerization	1
Alternative Carbon Source	7
Anaplerotic Reactions	1
Cell Envelope Biosynthesis	29
EMP Pathway	5
Membrane Lipid Biosynthesis	16
Pentose Phosphate Pathway	4
Pyrimidine Biosynthesis	1
Respiration	5
Salvage Pathways	17
Transport	36
	122

Fig. 27

COMPARISON OF MINIMAL METABOLIC GENE/REACTION SETS BASED ON FUNCTIONAL CLASSIFICATION*

Metabolic Function	Essential Gene Set+ *Ref. (2)*	Minimal Gene Set *Ref. (5)*	Minimal Reaction Set
	# Genes	# *Genes*	# Reactions
Amino acid biosynthesis	0	0	1
Biosynthesis of cofactors, prosthetic groups, and carriers	4	3	0
Cell envelope	2	11	29
Central intermediary metabolism	7	7	1
Energy metabolism	31	32	21
Fatty acid and phospholipid metabolism	5	7	16
Purines, pyrimidines, nucleosides, and nucleotides	17	14	18
Transport and binding proteins	17	25	36
	83	**99**	**122**

Fig. 28